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Preliminary Studies of the Effects of Sulfonamides on Fish and *Bacterium salmonicida.*

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INTRODUCTION.

Very little is known about the effects of sulfonamides on fish and their use in the prevention and treatment of bacterial and other parasitic diseases of fishes kept in captivity. Gutsell (1946, 1947) and Wolf (1947) have reported on the effectiveness of certain of these drugs on furunculosis, a fatal hemorrhagic disease of salmonoid fishes in aquaria and hatcheries, caused by *Bacterium salmonicida.* Both of these investigators found that best results were obtained with sulfamerazine. Weighed amounts of the drug per body weight or weight of food per day were given to fishes kept in hatcheries. By these methods the mortality due to furunculosis was rapidly and substantially decreased.

None of these experiments indicated whether the drugs used were prophylactic or curative in action. The present contribution deals with the toxic effects of sulfonamides on an aquarium fish, *Tilapia macrocephala* (Bleeker), and the action of these drugs on cultures of *Bacterium salmonicida* Lehmann and Newmann.

The writers wish to thank Dr. Mark Welsh, Lederle Laboratories Inc., for the sulfonamides and other chemicals used in these experiments and Professor William B. Sarles of the College of Agriculture, University of Wisconsin, for the culture of *Bacterium salmonicida.*

MATERIALS AND METHODS.

A. Bacteriological.

The drugs used were the sodium salts of sulfathiazole, sulfadiazine, sulfamerazine and sulfamethazine (Sulmet). Sterile tubes containing 0.8, 0.4, 0.2, 0.1 and 0.05% of the drugs in 5 cc. of nutrient broth were inoculated with *Bacterium salmonicida* (strain M-39) from an 18-hour-old broth culture. The degree of turbidity of each tube after 48 hours of incubation at room temperature was used as an indication of the amount of growth that had taken place. Tubes which showed slight or no apparent growth were subcultured on nutrient agar slants and kept at room temperature for 24 hours.

B. Toxicological.

The toxic levels were obtained by placing the fish in various concentrations of the sulfonamides and observing their reactions over a period of time. The experimental fish, *Tilapia macrocephala,* was chosen because of its hardiness and availability. The specimens varied from 2½ to 3 inches in total length. For this initial test two-gallon tanks containing 5 liters of conditioned water were used. One fish was placed in each tank and a specific amount of sulfonamide was added. The temperature was maintained at about 75° F. and the pH was checked frequently.

To test the toxicity of the various drugs with more than one fish per tank, a second series was started in which five fish were placed in twenty-gallon tanks containing 62 liters of conditioned water and to each of which sulfonamide was added to make up a concentration of 0.80 mg. per cc. Bratton and Marshall's (1939) method, employing a photoelectric colorimeter, was used to determine the amount of sulfonamide present per cc. Because of the high concentration of drug the tests were made on a ¼ cc. of the water. The pH was checked with each reading.

A third series was used to check the changes, if any, in the level of the nontoxic concentrations of sulfathiazole in a tank containing a single *Tilapia.* The experiment was extended for 120 hours.

A control tank was maintained under the same conditions for each of the experimental series. The pH remained at 7.8 for the duration of the experiment (30 days) and only one death was recorded.

RESULTS.

A. Bacteriological.

The tubes of broth containing 0.1% or greater concentrations of sulfathiazole and

[1] Based on material submited in partial fulfillment of the requirements for the Master of Science degree at New York University.

sulfadiazine showed no apparent growth. Sulfamerazine showed growth in tubes containing less than 0.2%. Growth occurred in tubes containing Sulmet at all concentrations. The results are tabulated in Table I.

TABLE I.

Action of various concentrations of sulfonamides on *Bacterium salmonicida.*

DRUG	% OF DRUG					
	0.8	0.4	0.2	0.1	0.05	Control
Sulfathiazole	O	O	O	O	XX	XXX
Sulfadiazine	O	O	O	O	X	XXX
Sulfamerazine	O	O	O	XX	XX	XXX
Sulmet	X	XX	XX	XX	XX	XXX

O, no growth; **X**, weak growth; **XX**, moderate growth; **XXX**, maximum growth.

Inoculation from the tubes which showed slight or no apparent growth were made on agar slants. These sub-cultures showed growth in all cases after a period of incubation of 24 hours at room temperature, indicating that the action of the drugs at these concentrations was bacteriostatic. The results are summarized in Table II. The symbols used to indicate the intensity of growth are the same.

TABLE II.

Results of subcultures from bacteriostatic growth of *Bacterium salmonicida.*

DRUG	% OF DRUG				
	0.8	0.4	0.2	0.1	Control*
Sulfathiazole	X	X	X	XXX	XXX
Sulfadiazine	X	X	X	XXX	XXX
Sulfamerazine	XX	XX	XXX	XXX	XXX

* The control nutrient agar slants were inoculated from the control nutrient broth tube.

B. Toxicological.

The results of the first series of tests on a single fish in two gallon tanks are summarized in Table III.

As shown in the Table, the non-toxic concentration for sulfathiazole was 0.1%, for sulfadiazine and sulfamerazine it was 0.5%, and for Sulmet it was 0.14%.

In a second series, five fish were placed in twenty-gallon tanks containing 62 liters of conditioned water. The drug concentration was 0.8 mg. per cc. This concentration remained more or less constant throughout the experiment. For Sulmet, a toxic reaction was obtained in 16 days, all of the fish dying. The drug concentration was 0.76 mg. per cc.; the pH shifted from 8.4 to 7.7. A toxic reaction resulted in 11 days for sulfamerazine. However, the drug concentration and pH remained constant. A toxic reaction was obtained in 20 days for sulfadiazine. There was a slight drop in pH (7.8 to 7.4) but the drug concentration remained the same. No toxic effects were obtained with sulfathiazole. The fish were kept in the solution, which remained constant in its drug concentration, for more than 30 days. The pH shifted slightly downward from 8.2 to 7.9.

TABLE III.

Reaction of *Tilapia macrocephala* to sulfonamides.

DRUG	% SOL.	pH OF WATER	pH OF WATER PLUS DRUG	TIME OF DEATH
Sulfathiazole	2	—	—	2½ hrs.
	1	7.4	9.0	24 hrs.
	0.5	8.0	8.5	3 hrs.
	0.25	7.4	8.0	24 hrs.
	0.1	7.3	8.0	non-toxic
Sulfadiazine	2.5	7.4	8.3	1½ hrs.
	2	—	—	48 hrs.
	1	7.4	8.4	48 hrs.
	0.5	—	—	non-toxic
Sulfamerazine	2	—	—	4⅓ hrs.
	1	7.4	8.5	4 hrs.
	0.5	8.0	8.1	non-toxic
Sulmet	2	—	—	5/6 hrs.
	1	7.4	9.1	1-1/6 hrs.
	0.5	—	—	1½ hrs.
	0.25	7.4	8.4	1½ hrs.
	0.14	8.0	8.9	non-toxic

Except for sulfathiazole, therefore, all of the other sulfonamides are toxic at this concentration.

A third series was studied to check the constancy of the drug concentration indicated above. A single fish in a two-gallon tank containing 5 liters of conditioned water was used. To these tanks sulfathiazole was added to make up a concentration of 0.8 mg. per cc. The results are tabulated in Table IV.

TABLE IV.

Concentration of sulfathiazole and pH at the start and termination of experiment (120 hrs.). One fish per tank.

Time in Hours		TANKS				
		1*	2	3	4	5†
Start	pH	8.3	8.4	8.5	8.0	8.5
	mg/cc	.69	.82	.82	.80	.80
24		8.3	8.5	8.4	8.5	8.5
		.68	.82	.81	.80	.80
48		8.2	8.2	8.1	8.1	8.5
		.69	.80	.80	.80	.80
96		7.8	7.8	7.8	7.8	8.0
		.70	.80	.82	.80	.80
120		7.8	7.8	7.9	7.9	8.1
		.70	.80	.80	.80	.80

* The lower concentration used in tank 1 was picked arbitrarily and has no significance except to indicate, as do the others, that the amount of drug present was the same throughout the experiment.

† Control tank, containing drug but no fish.

The common cause of fatality in all these experiments was hemorrhage apparent in the gills. Fishes kept in water with lethal concentrations of the drug appeared restless and attempted to jump out of the tank. As shown in Table III death occurred at various intervals. That pH was not a factor in these deaths can be seen from the fact that mortality also occurred in those tanks in which there was very little shift in the reaction. In addition, there were no deaths at the non-toxic levels even though there was a change in pH. Further studies on *Tilapia macrocephala* are being made on the effects of these drugs on the kidneys and other organs.

Conclusions.

Tests *in vitro* indicate that sodium sulfathiazole was most effective in producing a bacteriostasis of *Bacterium salmonicida.* The concentration which inhibited growth was also non-toxic for the test fish, *Tilapia macrocephala.* Since the drug concentration in the water remained approximately the same throughout the experiments, indicating that no absorption was taking place, it is believed that the sulfonamides were not acetylated. The liver of *Tilapia,* therefore, is like the liver of the dog in this respect (see Shay and co-workers, 1944). This may also be true for other teleosts. It seems that sodium sulfathiazole would be best used as a preventative rather than a curative drug for *Bacterium salmonicida.*

Summary.

A study was made of the effects of the sodium salts of sulfamerazine, sulfathiazole, sulfadiazine and sulfamethazine (Sulmet) on *Bacterium salmonicida,* the causative agent of furunculosis in salmonoid fishes. All four were found to be bacteriostatic in effect. Toxic reactions in the fish, *Tilapia macrocephala* (Bleeker), resulted from the use of three of the drugs in concentrations effective against *B. salmonicida.* Only sodium sulfathiazole proved non-toxic at a concentration that inhibited growth of this organism. There was no evidence that the fish absorbed the drug, since the concentration of the drugs in the water remained constant. It therefore appears that fish livers are unable to acetylate these compounds.

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